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Genetic parameters for predicted methane production and laser methane detector measurements[1]

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ABSTRACT: Enteric ruminant methane is the most important greenhouse gas emitted from the pastoral agricultural systems. Genetic improvement of livestock provides a cumulative and permanent impact on performance, and using high-density SNP panels can increase the speed of improvement for most traits. In this study, a data set of 1,726 dairy cows, collected since 1990, was used to calculate a predicted methane emission (PME) trait from feed and energy intake and requirements based on milk yield, live weight, feed intake, and condition score data. Repeated measurements from laser methane detector (LMD) data were also available from 57 cows. The estimated heritabilities for PME, milk yield, DMI, live weight, condition score, and LMD data were 0.13, 0.25, 0.11, 0.92, 0.38, and 0.05, respectively. There was a high genetic correlation between DMI and PME. No SNP reached the Bonferroni significance threshold for the PME traits. One SNP was within the 3 best SNP for PME at wk 10, 20, 30, and 40. Genomic prediction accuracies between dependent variable and molecular breeding value ranged between 0.26 and 0.30. These results are encouraging; however, more work is required before a PME trait can be implemented in a breeding program.

Key words: dairy cattle, genomewide association, genomic selection, heritability, methane

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INTRODUCTION

Enteric ruminant methane is the most important greenhouse gas emitted from the pastoral industry. The agricultural sector contributed 4.6 billion t of CO_2 equivalent [$GtCO_2$–eq]/yr in 2010 to global greenhouse gas emissions, of which enteric ruminant fermentation contributed 2 $GtCO_2$–eq/yr (Tubiello et al., 2013). Dairy cattle were the second highest source of enteric methane (19.44%) behind nondairy cattle at 56.04% (FAOSTAT, 2013). Methane production represents an energy loss for ruminants with 2 to 12% of the GE intake being lost as enteric methane (Blaxter, 1962; Johnson and Johnson, 1995). This arises from feed intake and composition, fermentation of feed including passage rate and rumen volume, the physiological state of the animal, and variation between individual animals (Hristov et al., 2013a,b; Goopy et al., 2013; Pinares-Patiño et al., 2013). Therefore, there are various mitigation strategies to reduce methane emissions per animal and per unit of production. These include improved productivity and efficiency of the animal, reduced culling at herd or flock level, change in feed type, use of supplements, immunization against methanogenic archaea, and direct selection on methane trait for genetic improvement (Martin et al., 2010;

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Wall et al., 2010). Of these, genetic improvement has a cumulative and permanent outcome.

High-density SNP panels have SNP selected to be equally distributed across the genome and all potential QTL are captured due to linkage disequilibrium with at least 1 of the SNP (Meuwissen et al., 2001). The ability to genotype thousands of SNP in single SNP panels has been used to map Mendelian traits, investigate patterns of linkage disequilibrium, map QTL, investigate domestication, and implement genomic selection for genetic gain (Kijas et al., 2009). Genomic selection was proposed by Meuwissen et al. (2001), where estimates are calculated on the effect each SNP has on a trait; the sum of the SNP effects can then be used to predict the animals' genetic worth. Combined with EBV, an overall genomic breeding value can be generated, which could potentially lead to doubling the rate of genetic gain already achieved through using EBV calculated from phenotypic data alone. The greatest benefits are for those traits that are hard to measure, measured late in life, or sex limited.

Enteric methane is an exemplar of a difficult trait to measure and methane emissions are not routinely measured as part of day to day management. A recent review by Pickering et al. (2013) stated that although there has been some research in quantifying methane emissions in certain environments, there is still significant work required before selection for reduced methane emissions could be incorporated into breeding programs. Measurement of enteric methane is costly using current respiration chamber techniques, but identifying a low-cost measurement technique or appropriate proxy still requires an initial evaluation against existing protocols. There is also insufficient knowledge about the correlations between predictor traits and methane emissions. Finally, the knowledge around genetic relationships between methane and production traits is incomplete.

The aim of this study was to calculate predicted methane emission (**PME**) trait in British dairy cows and investigate its genetic relationship with key production traits. Laser methane detector (**LMD**) data was available for a subset of the animals and the correlation between PME and laser data was also investigated. Finally, using EBV across the lactation, a genomewide association study (**GWAS**) was undertaken to identify genomic regions that may be associated with methane emissions. To undertake this work, the predicted methane equation from de Haas et al. (2011) was used.

MATERIAL AND METHODS

Blood sample collection was conducted in accordance with U.K. Home Office regulations (PPL number 60/4278 Dairy Systems, Environment and Nutrition) and procedures were approved by the Scotland's Rural College (**SRUC**) Animal Experimentation Committee. Otherwise, the study was restricted to routine on-farm observations and measurements that did not inconvenience or stress the animals.

Data

Data on 1,726 animals were obtained from the Langhill herd records collected since 1990 and has been well documented (e.g., Pollott and Coffey, 2008; Bell et al., 2010). The herd was located on the University of Edinburgh Langhill farm (Edinburgh, East Scotland, UK) until 2001 when it moved to the SRUC Dairy Research Centre on Crichton farm (Dumfries and Galloway, South West Scotland, UK) with data collection recommencing in 2002 after a period of acclimatization. The Langhill herd consisted of 2 genetic lines of cows selected for either weight of fat plus protein milk solids (**S]**) or selected to remain close to the average U.K. genetic merit for fat plus protein (**C**). During the study period, the cows were housed together and cows from the C and S lines were divided into either a high or low concentrate–based diet (feed group). Before 2001, this was approximately 1,500 and 2,500 kg concentrate per lactation for the low (n = 360) and high (n = 394) group, respectively. From 2002, the herd was divided into 2 contrasting management systems, a high-forage (**HF**) and low-forage (**LF**) system. In the HF system (n = 316), the cows grazed when sufficient herbage was available and were fed a complete diet containing between 70 and 75% forage in the DM when grass heights fell below set values and in the winter months. In the LF system (n = 305), the cows were housed throughout the year and were fed a complete diet containing between 45 and 50% forage in the DM. The LF diet contained approximately 1,200 kg concentrate while the HF diet contained approximately 3,000 kg concentrate in a year. The forage component of the complete diet consisted of grass silage, maize silage, and whole crop (Chagunda et al., 2009).

The data included daily milk yield (**MY**; 1,431,949 observations) summed from daily morning and afternoon milking while on the Langhill farm and 3 times a day milking on the Crichton farm. Protein, fat, and lactose milk composition was measured weekly. Live weight (**LW**) and BCS were measured weekly across the lactation (or average LW across the week based on weights 3 times a day after milking on the Crichton farm) and daily feed intake measures were undertaken for 3 to 4 consecutive days a week. Feed intake was measured through Calan Broadbent gates (before and including 2001) or HOKO automatic gates (after

2001). Morning, middle, and afternoon MY and feed intake measurements deviating by more than 3 SD from the average in that lactation were discarded.

The models formulated by Coffey et al. (2001) were used to estimate the amount of energy consumed and required for maintenance in megajoules per day. This was converted into daily DMI (kg DMI/d) consumed and required for maintenance by dividing by the ME of the feed used.

Predicted Methane Emission

Estimation of a PME was centered on the equation generated by de Haas et al. (2011), which was modified and calculated for each animal as follows: PME (g/d) = feed intake (kg of DM/d) × 18.8 (MJ/kg DM) × 0.06 × [1 + 0.018 × (level of intake – 1)]/0.05565 (MJ/g).

The equation by de Haas et al. (2011) was altered for a British fed dairy cow rather than a Dutch system, that is, the average GE of ruminant diets was taken as 18.8 MJ/kg DM and the correction for the level of feed intake as a multiple of maintenance was 1 + 0.018 × (level of intake – 1) (Alderman and Cottrill, 1993). The energy generated by methane of 0.05565 MJ/g (IPCC, 2006) and the methane production level (MJ/d) of 0.06 × GE intake as recommended by the Intergovernmental Panel on Climate Change (2000) for dairy cattle in developed countries remained the same.

Predicted methane emission was calculated daily and then averaged for each week of lactation. Predicted methane emission for lactations 1 to 5 and wk 1 to 44 were used to estimate genetic parameters.

Laser Methane Detector

Laser methane detector data was available on 57 cows born from 2004 to 2009 and was measured in 2010, 2011, and/or 2012. A point measure in mg/kg per meter was taken every 0.5 sec within a period of 1 to 5 min up to 3 times a day, 30 min apart, on 3 different days (Chagunda et al., 2013). Cows were randomly measured either in early, mid, or late lactation. Measurements were done at a distance of 1 m from the cows nostrils.

Each measurement of 1 to 5 min shows a cyclic pattern of troughs and peaks, with the peaks reflecting bouts of eructation. The peaks within a period were identified and the average of each peak was taken as the period emissions in mg/kg. Then, where possible, average daily methane emission for each week of lactation was estimated for each cow measured.

Statistical Analysis

Variance component estimation was performed fitting a bivariate model. The bivariate model between LMD data and PME consisted of a mixed linear model for LMD data and a random regression animal model for PME using ASReml (Gilmour et al., 2009). The model for LMD data was

$$y_{ijkl} = lac_i + myr_k(wk_j) + animal_l + e_{ijkl},$$

in which lac_i refers to the effect of the lactation *i,* $\text{myr}_k(\text{wk}_j)$ is the interaction between year of measurement *k* and *j*th week in lactations, animal*l* is the random effect of *l*th animal, and e_{ijkl} is the random error term. The random regression model fitted for PME was

$$y_{tijk} = F_i + \sum_{k=0}^{2} \text{Ø}_{jtk}\beta_k + \sum_{k=0}^{2} \text{Ø}_{jtk}u_k + \sum_{k=0}^{2} \text{Ø}_{jtk}p_k + e_{tijk},$$

in which F_i refers to the fixed effects of genetic groups, feed groups, Holstein percentage, year of calving by month of calving, and linear and quadratic effects of age at calving; β*k* are the fixed regression curves from fitting polynomials of order 2 on weeks of lactation nested within lactation number; u_{jk} and p_{jk} are second order polynomials for animal and permanent environmental effect, respectively; and e_{tijk} is the random error term.

Bivariate runs between PME with MY, DMI, LW, and BCS were also performed. The same random regression model fitted for PME was also fitted for MY, DMI, and LW in the bivariate analysis. The EBV were calculated for PME at wk 10, 20, 30, 40, and 44 from the PME–LMD bivariate analysis for the genomic analyses below. The bivariate results were used as LMD data may adjust PME beneficially, although it was hypothesized this would be minimal given the limited LMD data set. Software Mix99 (Lidauer et al., 1999) was used to calculate reliabilities for PME at wk 10, 20, 30, 40, and 44 and LMD data.

A separate bivariate between PME and LMD data was performed to calculate repeatability of measures across weeks within a lactation and across lactations. Fixed effects for both traits were the same as above. Animal and permanent environmental effects were fitted for both traits. The terms β_k, u_{jk}, and p_{jk} were not fitted for PME.

A pedigree file was constructed and consisted of 2,131 records. There were 157 sires and 923 dams with progeny.

Genotyping

High-quality genomic DNA samples were isolated from heparinized blood. Of the 2,131 animals available in the pedigree, 731 animals had been genotyped with

the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA) containing 54,001 SNP. Genotyping results were processed through a quality control pipeline before analysis. Animals were discarded if they had a call rate < 96% ($n = 27$) and SNP were discarded if they had a call rate < 97%, appear nonautosomal (including pseudoautosomal), had a minor allele frequency = 0, and were on chromosome X. This removed 5,044 of the 54,001 SNP genotyped.

Genomewide Association Analysis and Genomic Prediction

Dependent variables (y) of the 731 animals genotyped were calculated taking into account the individuals' own and descendants' information. Parent average effects were removed from the EBV using the method described by Garrick et al. (2009), assuming all genetic variation was explained by the markers ($c = 0$). The resulting values are deregressed by the reliabilities of their EBV with parent contributions removed.

Data was filtered on breed and reliability before analysis. Animals were discarded if they were less than 75% Holstein. Only those y values with reliabilities greater than or equal to 0.8 h^2 were used as phenotypes. There were 462, 461, 246, 432, and 402 y values for PME at wk 10, 20, 30, 40, and 44, respectively, used for genomic prediction and GWAS. For estimating accuracy of genomic prediction, individuals with y values were further assigned into training and validation sets based on birth year (Table 1); for GWAS, all animals (training and validation) were used in the analysis. The first birth year for validation animals was 2003. Analyses were performed using R software (R Core Team, 2013). To estimate SNP effects ($\boldsymbol{b_i}$), fixed heritabilities, obtained from the PME–LMD bivariate analysis, of 0.06, 0.06, 0.07, 0.13, and 0.17 for PME at wk 10, 20, 30, 40, and 44, respectively, were used assuming additive genetic variance (σ^2_u) was equal to the value used to calculate the EBV. The b_i were calculated using the genomic BLUP model, with a genomic relationship matrix (**G1**) of VanRaden (2008):

$$\mathbf{G1} = \mathbf{ZZ'}/[2\textstyle\sum p_i(1 - p_i)],$$

in which **Z** is the SNP matrix $-2p_i$, $1 - 2p_i$, and $2 - 2p_i$ for BB, AB, and AA, respectively, and pi is the frequency of the A allele of the ith SNP in the population. A linear mixed model was fitted to y, including the first 6 principal components (**PC**) of **G1** to account for population stratification. Animal effects distributed as $N(\mathbf{0}, \mathbf{G1}\sigma^2_u)$ was fitted as a random term and residual effects distributed as $N(\mathbf{0}, \mathbf{R})$, in which **R** is a diagonal matrix with diagonal elements $(1 - r^2)/r^2$, in which r^2 is the reliability of y.

Table 1. Number of animals genotyped on the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA) and assigned to training and validation sets for each trait

Trait	Training	Validation	Total no.
Predicted methane emission wk 10, g/d	227	235	462
Predicted methane emission wk 20, g/d	227	234	461
Predicted methane emission wk 30, g/d	108	138	246
Predicted methane emission wk 40, g/d	210	222	432
Predicted methane emission wk 44, g/d	191	211	402

Significant values for b_i were calculated assuming b_i followed a normal distribution with mean 0 and variance

$$\text{var} = [2p_i(1 - p_i)m \times varb_i]/\textstyle\sum[2p_i(1 - p_i)],$$

in which m is the number of SNP. The $-\log_{10}(P)$ values were graphed in a Manhattan plot using positions from bovine genome version 4.6. To account for multiple testing, a genomewide nominal significance threshold for type 1 error of less than 5% was approximated using the Bonferroni correction (Rice, 1989), 0.05/nSNP equal to P-value of 1.02×10^{-6} or $-\log_{10}(P)$ of approximately 6. This is stringent as it assumes that each SNP genotype is independent. A lower nominal threshold at $P < 0.001$ [$-\log_{10}(P) = 3$] was also used.

To estimate the accuracy of genomic prediction, molecular breeding values (**MBV**) were obtained by multiplying b_i by SNP genotype (BB = 0, AB = 1, and AA = 2) and summing overall SNP within an individual. Assuming that the effective heritability ($\boldsymbol{hg^2}$) is equal to the average r^2, prediction accuracy was estimated as $\text{cor}(y, \text{MBV})/h_g$, weighted by $1/(1 - r^2)$ using animals in the validation set.

RESULTS

A summary of the raw data is presented in Table 2. From the overall data set, there were 1,678 cows with 1,144,665 daily MY observations for wk 1 to 44 up to the first 5 lactations of their life. After calculating average daily measurements for each week of lactation, this equated to 177,019 observations for MY, 96,588 observations for DMI, and 67,304 PME observations. There were only 45 animals with PME and LMD measurements (total of 97 individual LMD observations) for the same weeks.

Milk yield followed the standard lactation curve, with a sharp increase in early lactation, reaching a peak of average daily MY of 35.14 kg/d in wk 6 and a subsequent gradual decrease to 20.43 kg/d at wk 44 of lactation (Fig. 1). Predicted methane reached a maxi-

Table 2. Number of animals, observations, mean, and SD for each trait

Trait	Cows	No. daily	No. weekly	Mean	SD
Milk yield, kg/d	1,678	1,144,665	177,019	28.50	8.88
Predicted methane emission, g/d	1,305	274,211	67,304	230.59	61.31
DMI, kg/d	1,337	376,849	93,588	16.33	4.36
Laser methane data, mg/kg	57	1,308	173	180.22	46.99
Live weight, kg	1,629	565,804	150,916	606.70	73.08
BCS	1,588	144,892	126,122	2.32	0.50

mum by wk 11, with an average daily PME of 245.30 g/d. The curve then stayed at around this level for the remaining weeks of lactation. Dry matter intake followed a curve similar to PME, with the curve settling out at a maximum of average daily DMI of 17.29 kg/d by wk 11. For LW, there was an initial drop of approximately 10 kg to average of 583.89 kg at wk 4 and then a gradual increase to an average LW of 647.22 at wk 44. For BCS, there was a decrease from average of 2.55 to 2.10 at wk 15 and then a gradual increase to an average BCS of 2.49 at wk 44.

There were limited LMD data, with animals randomly measured in either early, mid, or late lactation. There were no measurements available for wk 26, 28 to 38, and 41 (Fig. 2).

Genetic Analysis

A summary of heritability estimates for all traits plus genetic and phenotypic correlations with PME across lactation is shown in Table 3. These are represented graphically in supplementary material (Fig. 1–3). Heritability for PME was relatively stable across lactation (Fig. 3), with a mean heritability of 0.13 and peak heritability at wk 44 of 0.30. Genetic correlation was close to 1 with DMI across the whole lactation. This is as expected because the calculation of PME relies heavily on feed intake. The genetic correlation with MY followed the lactation curve for PME in Fig. 1 above, with a closer relationship at wk 44. For LW the genetic correlation slowly decreased as moved through lactation, with a final correlation at wk 44 of 0.62. For both BCS and LMD data, the genetic correlation started as positive with peak correlation at wk 4 and 14, respectively; the correlation became negative by wk 18 and 33, respectively. The phenotypic correlation between PME and DMI and BCS was relatively flat across lactation. For MY, phenotypic correlation increased and became relatively stable by week 12 (r_p = 0.59–0.58); the phenotypic correlation started to decrease at wk 32. For LW, the phenotypic correlation decreased slowly through lactation.

A separate bivariate between PME and LMD data, fitting a repeatability model (instead of a random regression model) fitted for PME, was performed to estimate repeatability of weekly measures within and across lactations. Heritability for PME and LMD data was 0.06 ± 0.02 and 0.11 ± 0.16, respectively. Repeatability within a lactation was 0.53 ± 0.01 and 0.07 ± 0.08, respectively, and across lactations was 0.38 ± 0.01 and 0.03 ± 0.08, respectively.

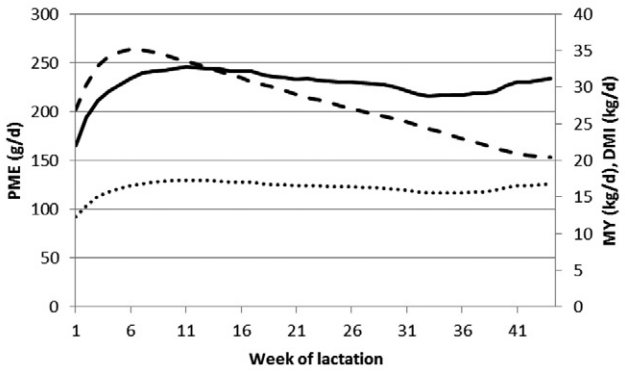

Figure 1. The lactation curves for predicted methane emission (PME; g/d; solid line), milk yield (MY; kg/d; dashed line), and DMI (kg/d; dotted line) up to wk 44 of lactation.

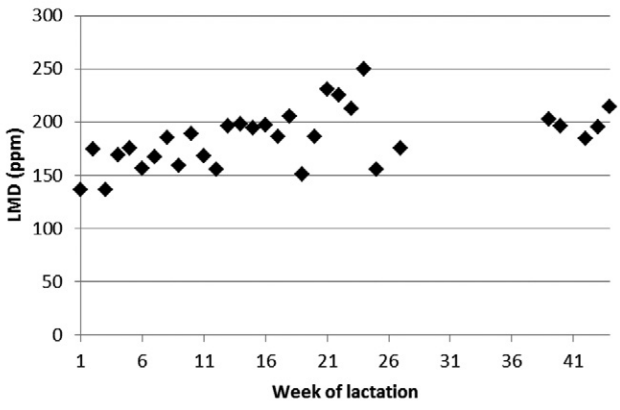

Figure 2. The lactation curve for laser methane detection (LMD) data (mg/kg) up to wk 44 of lactation.

Table 3. Minimum, maximum, and mean values for heritability (h^2) and genetic (r_g) and phenotypic (r_p) correlations for all traits for wk 1 to 44 of lactation in bivariate ASReml analysis with predicted methane emission (PME; g/d)

	h^2			r_g			r_p		
Traits[1]	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
PME	0.05 ± 0.03	0.30 ± 0.06	0.13 ± 0.04						
DMI	0.05 ± 0.03	0.28 ± 0.05	0.11 ± 0.03	0.99 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
MY	0.20 ± 0.03	0.35 ± 0.04	0.25 ± 0.04	0.42 ± 0.10	0.71 ± 0.17	0.55 ± 0.12	0.41 ± 0.01	0.59 ± 0.02	0.55 ± 0.01
LW	0.89 ± 0.03	0.93 ± 0.03	0.92 ± 0.03	0.62 ± 0.03	0.93 ± 0.08	0.84 ± 0.05	0.40 ± 0.02	0.53 ± 003	0.49 ± 0.02
BCS	0.25 ± 0.03	0.46 ± 0.05	0.38 ± 0.04	–0.11 ± 0.16	0.44 ± 0.20	0.07 ± 0.18	0.07 ± 0.02	0.11 ± 0.03	0.09 ± 0.02
LMD data			0.05 ± 0.07	–0.50 ± 0.78	0.59 ± 0.98	0.21 ± 0.90			

[1]DMI is expressed in kilograms per day; MY = milk yield (kg/d); LW = live weight (kg); LMD = laser methane detector (mg/kg).

Genomewide Association Analysis

Population Structure. Principal components were fitted to account for any population stratification. The first 6 PC (Table 4) were sufficient to account for approximately 40% of the genetic variation contained in the genomic relationship matrix. The first 2 PC calculated for all animals that were genotyped and had breeding values for PME traits (n = 647) are shown in Fig. 4. The first PC separated the 2 selection lines (C and S) while the second PC further separated the control line animals by familial relationships.

Association Analysis. The quantile–quantile plots (Supplementary Fig. 4) showed that the deviation of the observed $-\log_{10}(P)$ values from the expected values was insignificant (lambda ranged between 0.99 and 1.04). The SNP seen to be deviating from (were higher) the expected values (0–1 line) were interpreted as SNP associated with the trait of interest, as the SNP are departing from the null hypothesis of no genetic association and no linkage disequilibrium between SNP. There were 35, 41, 33, 38, and 39 SNP that reached the nominal genomewide significance threshold of $P < 0.001$ for PME at wk 10, 20, 30, 40, and 44, respectively.

There were 8 SNP above a threshold of $-\log_{10}(P)$ of 4 for PME at wk 10, 20, 30, 40, and 44 on BTA4 (rs41591564 and rs110492803), BTA5 (rs42384792), BTA7 (rs110349600), BTA11 (rs41620644), BTA15 (rs41763705), BTA22 (rs110837838), and BTA23 (rs109661590; Fig. 5A–5E; Supplementary Table 1). There was 1 SNP (rs110349600) located on BTA7 that was within the best 3 SNP [$-\log_{10}(P)$ between 4.12 and 4.45] for PME at wk 10, 20, 30, and 40 (was rank 10 for PME at wk 44). The minor allele frequency (B allele) was 0.15 and the average SNP effect was –0.43 g/d (range –0.62 to –0.16 g/d for each extra A allele). There were 2 genes within 100 kb of this SNP: the *RUN and FYVE domain containing 1* (*RUFY1*; Online Mendelian Inheritance in Man [**OMIM**] number 610327) and *Heterogenoeous Nuclear Ribonucleoprotein H1* (*HNRNPH1*; OMIM number 601035). The region also overlapped 10 QTL previously reported in the literature, including QTL for fat thickness at the 12th rib, marbling score, milk β-casein percentage, milk fat percentage, milk fat yield, maternal weaning weight, birth weight, dystocia, scrotal circumference, and stillbirth.

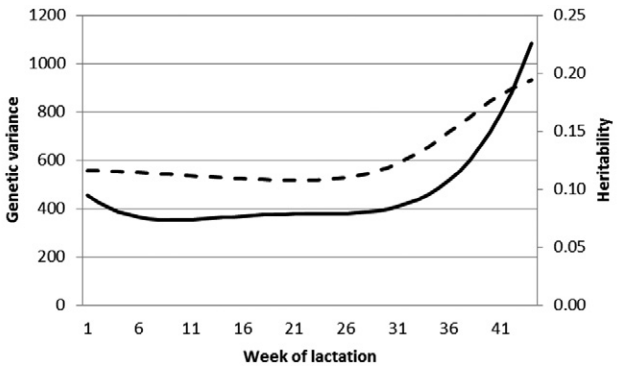

Figure 3. Genetic variance (solid line; SE range 95.80–312.82) and heritability (h^2; dashed line; SE range 0.03–0.05) of predicted methane emission for each week of lactation, averaged across all bivariate runs.

The Pearson correlation between the SNP $-\log_{10}(P)$ values for the traits ranged between 0.87 and 0.93 when between 2 immediate traits, that is, PME at wk 10 and 20, but decreased as time increased between the 2 traits, that is, 0.60 for PME at wk 10 and 44 (Table 5).

Genomic Prediction

The accuracies of the 6 traits calculated as the weighted accuracy of the correlation between MBV and y were 0.29, 0.30, 0.28, 0.30, and 0.26 for PME at wk 10, 20, 30, 40, and 44, respectively. The accuracies were compared to the theoretical accuracies using Eq. [8] from Goddard (2009), following the assumptions of an effective population size of 99 (Table S1 in The Bovine HapMap Consortium, 2009), number of animals available per trait, and genome length of 30 M. The theoretical accuracies were 0.15, 0.14, 0.08, 0.14, and 0.11 for PME at wk 10, 20, 30, 40, and 44, respectively. The accuracy estimates obtained in this study are higher than those theoretically

Table 4. The breed genotypic variance explained by the first 6 principal components (PC) for each trait

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Predicted methane emission wk 10, g/d	0.19	0.07	0.06	0.04	0.03	0.03
Predicted methane emission wk 20, g/d	0.19	0.07	0.06	0.04	0.03	0.03
Predicted methane emission wk 30, g/d	0.18	0.07	0.06	0.04	0.04	0.03
Predicted methane emission wk 40, g/d	0.18	0.07	0.06	0.04	0.04	0.03
Predicted methane emission wk 44, g/d	0.19	0.07	0.06	0.04	0.04	0.03

calculated. The reason for the higher accuracies is the theoretical values are for "unrelated" animals, that is, approximately 10 generations or more distant. In this study, the validation animals were 5 yr removed, at maximum, from the youngest animals in the training set and therefore have higher estimated accuracies as expected from theory. In practice, the validation animals would have an ancestor 1 to 3 generations distant in the training data set.

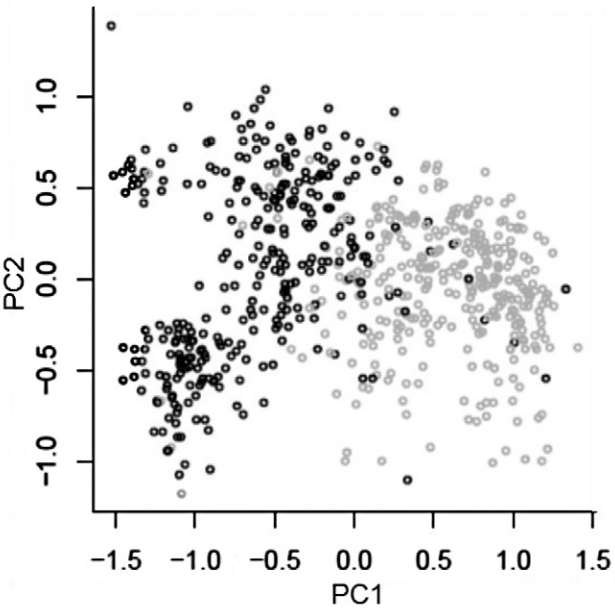

Figure 4. First 2 principal components (PC) calculated for all animals with genotypes, separated by genetic line controls (black) and selected for kilograms of fat plus protein (gray).

DISCUSSION

The objective of this study was to evaluate the use of PME in a British dairy data set and whether this trait could be used for genomic selection. Enteric CH_4 emissions are not routinely measured within production systems; therefore, for genetic improvement, either an industry reference herd requires complete phenotyping for CH_4 to establish a training set for genomic prediction or, alternatively, a proxy is developed, which can be used for genetic selection. The work of de Haas et al. (2011) has previously investigated the use of PME as a proxy for dairy cows. We modified their equation to suit a British fed dairy cow and combined it with laser methane detection data in a bivariate run to produce estimated breeding values for PME for subsequent use in a GWAS.

The limitations of the PME equation are well described by de Haas et al. (2011). These included that the equation relies on the variation in feed intake of the animals; uses the Intergovernmental Panel on Climate Change Tier 2 method, which is not suitable for differing diets (although only 2 different diets were used here, high or low concentrate; and was taken into account when calculating daily DMI using equations from Coffey et al., 2001); and assumes CH4 emission per unit feed GE is constant and independent of the variation in animals' ability to ferment its feed. Methane conversion rates (**Ym**) are not varied in the equation and therefore this predicted measure can capture only the variation in methane emissions due to feed efficiency and not any potential variation that exists in the methane conversion factor. Furthermore, it is not the same as measuring methane itself and we were limited in the data to accurately assess the correlation between PME and LMD data.

The low repeatability of LMD data is likely due to the paucity of the data for LMD data. There is also limited data on the relationship between LMD data and respiration chambers, which are considered the reference method for methane measurement. For dairy cattle, correlation coefficients were 0.80 and 0.47 for an indirect open-circuit and an open-circuit respiration chamber, respectively (Chagunda and Yan, 2011; Chagunda et al., 2013). Until it can be demonstrated that LMD data is repeatable in the current context and additional data is available, it is not recommended that LMD data be used as a measurement of methane emissions in genetic selection programs. Therefore, only GWAS and genomic prediction results for PME are presented here. The PME equation relies heavily on DMI, as seen by the close to unity genetic and phenotypic correlations of PME to DMI. This relationship was also seen by de Haas et al. (2011). The PME equation does not allow for varying Ym of the animal. The initial hope of this study was that the LMD data could be used to beneficially modify the PME EBV estimates to take account of the heavy influence on DMI. Due to the limited LMD data set, this was not achieved and the correlation between PME EBV from the univariate and bivariate analyses ranged between 0.99 and 1.00.

Due to the high correlation between PME and DMI, this is equivalent to selecting for altered residual feed

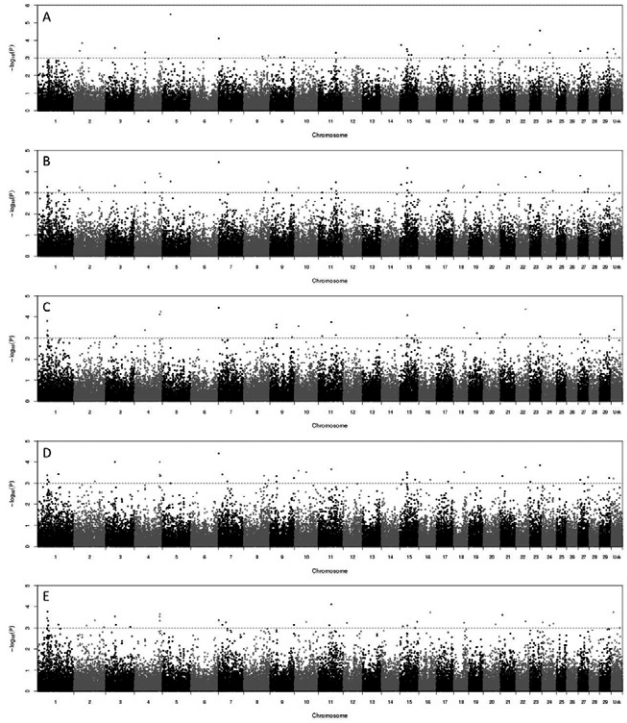

Figure 5. Manhattan plot of $-\log_{10}(P)$ values of SNP for predicted methane emissions at 10 (A), 20 (B), 30 (C), 40 (D) and 44 (E) wk. Ordered on the bovine genome version 4.6 map. The dash line represents $P < 0.001$ significance.

Table 5. Pearson correlations (±SE) between the –log10(*P*) values for predicted methane emissions (PME) at wk 10 (PME10), 20 (PME20), 30 (PME30), 40 (PME40), and 44 (PME44)

Trait	PME20	PME30	PME40	PME44
PME10	0.87 ± 0.002	0.72 ± 0.003	0.69 ± 0.03	0.61 ± 0.004
PME20		0.93 ± 0.002	0.80 ± 0.003	0.64 ± 0.004
PME30			0.89 ± 0.002	0.74 ± 0.003
PME40				0.92 ± 0.002

intake (**RFI**). Fitzsimons et al. (2013) and Pickering et al. (2013) have looked at the relationship between RFI and methane and suggest that selection for improved RFI would reduce methane emissions. Fitzsimons et al. (2013) indicated a decrease in methane by 23 g/d was associated with each 1 kg DM/d decrease in RFI. Expressed in percentages, this means a 10% decrease in DMI (kg DM/d) would result in a 6.1% decrease in methane (g/d). Work by Basarab et al. (2012) and expanded in Pickering et al. (2013) suggested that the carbon intensity of 4 different calf- and yearling-fed beef production systems would reduce by 8.85%, compared to baseline scenario, when there was a 10% reduction in DMI (equivalent to 10% improvement in feed efficiency) via selection for low RFI. In summary, selection for RFI slightly increases methane emissions per unit of DMI but decreases overall daily emissions per animal.

One SNP on chromosome 7 was identified to be likely associated with PME, as it appeared in the top 3 SNP for the traits PME at wk 10, 20, 30, and 40. There were 2 genes within 100 kb of this SNP, *RUFY1* and *HNRNPH1*. *RUFY1* is expressed in brain, kidney, liver, lung, placenta, and testis and participates in early endosomal membrane trafficking (Yang et al., 2002). *HNRNPH1* belongs to a set of polypeptides that bind heterogeneous nuclear RNA, produced by RNA polymerase II (Masuda et al., 2008). The heterogeneous nuclear ribonucleoprotein H-binding motif "UGGG" is overrepresented close to the 3′ end of introns, causing alternative splicing of the downstream exon. There has been little previous research on identifying regions associated with methane emissions. As part of their study on PME, de Haas et al. (2011) performed a GWAS on cumulative PME from wk 1 to 30. There were no similarities between that study and the results presented here. A study in sheep involving the Ovine SNP50K bead chip identified peaks near Tetraspanin14 (9) and Peroxisomal Biogenesis Factor (OAR 15) for gross methane (g/d) and methane yield (g/kg DMI), respectively (Rowe et al., 2014). However, these regions did not reach Bonferroni significance. It is unclear how the 2 genes identified in this study could be involved in methane emission or, alternatively, they simply reflect differences in intake. However, the regions that reached –log10(*P*) value of 4 are tabulated here (Supplementary Table 1) so that future researchers can combine these results in meta-analyses.

The accuracy between MBV and *y* for the PME traits ranged between 0.26 and 0.30. This is approximately equivalent to a measurement on an individual animal. These accuracies are similar to the accuracy between predicted breeding value and true breeding value obtained by de Haas et al. (2011) for PME (0.37 and 0.21 for direct genomic values and EBV, respectively) and slightly lower than those obtained by Verbyla et al. (2010) for energy balance (0.52 and 0.37 for direct genomic values and EBV, respectively). Accuracies for gross methane and methane yield in sheep data set were 0.37 and 0.43, respectively (Rowe et al., 2014). These are realistic starting values, given the limited data set and the accuracy of the measurement technique.

Methane emissions are a difficult trait to measure. Currently, there are numerous ways to measure the methane emission trait or its proxies. However, use of different measurement techniques generates individual data sets that are hard to combine given our current poor knowledge of repeatability and heritability of these traits and their genetic correlation with each other. An international initiative is currently underway devising

strategies to measure methane that would enable international meta-analysis (Pickering et al., 2013). The data and results presented here are an attempt to provide 1 such data set. It currently could be combined with the data from de Haas et al. (2011) but both these data sets need a robust phenotypic and genetic correlation with actual methane emissions to be combined with data sets where these traits are measured directly. This includes knowledge about how these rankings change with feeding level with 2 feeding levels offered in the current study while the study of de Haas et al. (2011) was ad libitum. The LMD measurements undertaken as part of this study were insufficient both in number and frequency to derive these relationships.

Conclusions

This study has looked at using PME and LMD data both for genetic parameter estimates and for potential use for genetic prediction. The current quantity of data from the LMD data is not suitable for genomic prediction. Predicted methane emission has been shown to be heritable and can be predicted via genomic selection with moderate accuracy and therefore could be used. However, its validity depends on the use of phenotypic equations derived from altering food intake in a small number of individuals to derive a methane feed intake relationship and has implicit assumptions that this same relationship holds for both phenotypic and genetic relationships for these traits when animals are fed under a variety of conditions. Additional work is required to validate these predictions.

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